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ABSTRACT

A recurring mortality associated with infectious hematopoietic necrosis (IHN) in outmigrating sockeye salmon, *Oncorhynchus nerka*, was investigated in 1982, 1983, and 1984 at a smolt-enumeration weir at Hidden Creek, Alaska. From 0.2% to 0.3% of the smolts trapped at the weir were affected.

Cutaneous lesions on the caudal peduncle and tail (the most frequently seen signs of disease) were often associated with infectious hematopoietic necrosis virus (IHNV). These signs in live or morbid fish might be useful as a means to sort infected smolts. In addition, 2.2% of the healthy-appearing, randomly sampled smolts were infected with the virus. There were no significant differences in virus titers between pooled kidney, liver, and spleen tissues and tissue from caudal peduncle lesions from the same fish or between the affected live and dead fish. Virus intensity ranged from 10^4 to 10^7 plaque-forming units (pfu) per gram. The high concentrations of virus within caudal peduncle lesion tissues were not likely to be incidental from the presence of the virus in the water. The geometric mean IHNV titers in sockeye salmon ovarian fluids were within one $\log_{10}$ at 10^4 to 10^5 pfu/g for 1981, 1982, and 1983. Prevalences of IHNV in ovarian fluids of spent sockeye salmon were 26.8% to 100%.

KEY WORDS: Infectious hematopoietic necrosis, sockeye salmon, *Oncorhynchus nerka*, viral epizootics.

INTRODUCTION

From 1980 through 1986, the viral disease, IHN, was associated with annual mortalities of sockeye salmon smolts at Hidden Creek (Burke and Grischkowsky 1984) in the Kenai River system. In the first report of a naturally occurring IHN epizootic in sockeye salmon, the

virus was not detectable in fish larger than fry, even when those fish were followed through the smolt migration (Williams and Amend 1976). The detection of this condition in 1- and 2-year-old sockeye salmon smolts at Hidden Creek (the outlet of Hidden Lake) was unique, as reported by Burke and Grischkowsky (1984). Historically, the acute form of IHN had not been associated with smolt-aged fish; the pathogen, IHNV, usually has been detected only among diseased fry and spawning adults. An outbreak of IHN, however, in a feral population of 2-year-old kokanee (*O. nerka*) was described by Traxler (1986). Many dead and live smolts at Hidden Creek had large cutaneous lesions on the caudal peduncle. The involvement of IHNV in the mortality was determined, and signs of the disease were also found in normal-appearing smolts when tissues were microscopically examined. Yasutake (1978) found that yearling sockeye salmon with IHN frequently lacked many of the typical signs of the disease.

Previously, Carlisle et al. (1979) associated cutaneous hemorrhages with IHN-infected rainbow trout, *Salmo gairdneri*, fry. Rucker et al. (1953), in an episode later confirmed to be IHN, found sockeye salmon about 10 cm in length and 8 months old to have external hemorrhagic areas. Fungus that was present on about half the fish was thought to be only an indicator of physiologically weakened fish and necrotic tissue.

High densities of sockeye salmon and low water flows were conducive to fish-to-fish transmission of IHNV during an IHN epizootic (Mulcahy et al. 1983a). Gills were noted as having high concentrations of IHNV and were thought to be portals of IHNV entry (Mulcahy et al. 1983a). The Alaska Department of Fish and Game (ADF&G) decided to enhance this lake through supplemental fry plants.

A short history of Hidden Lake is useful. Preweir escapement estimates (1947-1971), made by aerial or boat surveys, did not exceed 3,700, and more accurate counts of adult returns were obtained following the establishment of a weir in 1971. But the highest escapement prior to enhancement returns did not exceed 5,800 fish. There are several reasons why Hidden Lake is spawning-area-limited: (1) There is no inlet stream, (2) there is an absence of suitable habitat in the outlet stream, and (3) there is a predominance of steep and rocky

shores. Accordingly, this stock is exclusively a beach-spawning population. The sockeye salmon principally have a 4-year life cycle (Table 1). The lake has been enhanced with planted fry. The brood stock for these fry were from Hidden Lake. The eggs were incubated and fry reared at three hatcheries: (1) Crooked Creek Hatchery (on the Kenai Peninsula), 1977 and 1979; (2) Big Lake Hatchery (near Wasilla), 1978; and (3) Trail Lakes Hatchery (on the Kenai Peninsula), 1983, 1984, and 1985. The post-enhancement escapement increased to 27,448 in 1980, 15,939 in 1981, 27,832 in 1984, and 25,000 in 1985.

Because only a small number of fry were planted into Hidden Lake in 1979 and none were planted between 1980 and 1982, there were essentially only naturally produced smolts from 1980 through 1983; however, in 1984 an estimated 61.2% of the outmigrating sockeye salmon smolts were produced through enhancement. In order to monitor IHN occurrence during the enhancement, sampling was initiated. The specific external signs associated with IHN in sockeye salmon smolts were noted and an attempt made to detect IHNV in sockeye salmon smolts that were normal in appearance. Virus titers and prevalence were compared in several types of samples.

MATERIALS AND METHODS

Smolt Collection

From 1982 through 1984, abnormal live or dead sockeye salmon smolts were collected with fyke nets at Hidden Creek during June and July. Water temperatures were recorded at the site during smolt collection. Notations were made of any external abnormalities of smolts during 10 June through 3 July each year. Fish selected for viral assays were those with external hemorrhages or cutaneous lesions of the caudal peduncle; 14, 79, and 35 smolts were evaluated using the viral-plaque assay (Burke and Mulcahy 1980) in 1982, 1983, and

Table 1. Numbers of sockeye salmon adults into the lake from 1976 through 1985, at Hidden Lake, listed by brood year. Three zeros indicate estimates.

BROOD YEAR	FRY PLANTED	SMOLT OUT-MIGRATED	RETURN YEAR									
			1976	1977	1978	1979	1980	1981	1982	1983	1984	1985
1972			4,860									
1973				1,055								
1974		29,638			4,647							
1975		20,870				5,762						
1976	311,753	111,466					27,448					
1977	301,279	94,349						15,939				
1978	8,256	81,942							9,790			
1979		161,522								11,297		
1980		222,673									27,832	
1981		235,000										25,000
1982	1,092,684	419,797										
1983	1,240,000	396,000										
1984	1,805,792											
1985												

1984, respectively, from numbers observed to be abnormal or dead of 668, 535, and 938. During the peak runs, subsamples were taken to estimate totals because there were too many fish coming through the net to count. Subsamples consisted of timed counts during periods of maximal migration. Disease-related samplings were conducted to monitor continued involvement of IHN with the smolt stage of this species and to examine viral intensity in organs from live and dead fish with caudal peduncle or caudal fin lesions. Dates of sampling were included to amplify Burke and Grischkowsky's (1984) observations that the mortality predominantly occurred during the latter part of the migration.

Additionally, random samples of normal-appearing fish were collected in 1984 to estimate disease incidence in the population. Then, approximately 15 fish were killed and placed in individual plastic bags at 12-hour intervals during two-day sampling periods (four samples) occurring every 7 to 10 days between 13 June and 3 July. A total of 181 smolts were collected in this manner.

Infected Adult Salmon

From 1981 through 1983, the IHNV infection among female adult sockeye salmon was evaluated each year by collection of 59 to 71 individual ovarian fluid samples from spawned fish, or those with eggs loose in the coelomic cavity. These fish were collected from the natural spawning grounds with a beach seine. All had completed spawning, except in 1981 when approximately half had spawned and half were still spawning. The collections were made on 10, 20, and 27 September 1981, 1982, and 1983, respectively.

Sample Processing

All samples were maintained at about 4°C in an ice chest or portable refrigerator for 1 to 4 days before transporting to the lab. They were processed either fresh within a total of 5 days or after a 4-day storage at -80°C. About half the 1983 smolt samples had an extra 45 days of storage at -80°C, and the 1984 smolts were held about 26 to 82 days at that temperature. The viral-plaque assays of stored samples were completed in 40 to 90 days after freezing.

Viral-plaque assays were conducted on these samples to determine IHNV prevalence and intensity. The concentration of IHNV in pfu per milliliter of ovarian fluid was determined for each female. These values were converted to $\log_{10}$ for subsequent calculation of geometric sample means. The plaque assay utilized *epithelioma papulosum cyprini* (EPC) cells, individual samples, 10^{-6} final sample dilution, and staining with 0.5% crystal violet in 40% formalin after 7 days incubation at 15°C (Burke and Mulcahy 1980). Spleen and kidney tissues taken from abnormal live and dead smolts in 1982 were combined in equal proportions; for comparison, brain and gill tissues from the same fish were independently tested. In 1983 and 1984, spleen, kidney, and liver tissues were pooled in equal portions from abnormal live and dead smolts; peduncle lesions were also sampled when present. Statistical comparisons of viral intensity were made on data converted to $\log_{10}$ so as to reduce disproportionate effects of large exponents. The resulting geometric mean titer values and their standard deviations were expressed.

RESULTS

1982

In 1982, 222,673 sockeye salmon smolts were estimated at the fyke net sampling site; of these, 668 (0.3%) abnormal or dead smolts showing signs were suspected of having IHN. Of these, 46 were captured and only 14 smolts tested for IHNV. Nine (64.3%) had virus present in the pool of spleen, kidney, gill, or brain tissues. Eight of the 9 infected smolts had IHNV in all three types of collected samples, while one had virus only in the gills and was not considered diseased at that time (Table 2). Geometric mean viral intensities for these infected sockeye salmon smolts were highest in pooled spleen and kidney tissues and lowest in brain tissues. When the external abnormalities were recorded during the last week of sample collection, the following results for the 46 affected fish showing lesions were

Table 2. Prevalence of infectious hematopoietic necrosis virus (IHNV) in pooled kidney, liver and spleen of sockeye salmon smolts for Hidden Creek in 1982-1984.

Year	Smolt Condition	IHN Prevalence	Geometric Mean Titer
1982	Live with signs of IHN	9/14[a] (63.3)[b]	4.2×10^3
	Live with lesions on peduncle or tail	33/46 (71.7)	ND
	Live without signs of IHN	ND	ND
	Dead with signs of IHN	ND	ND
	Dead without signs of IHN	ND	ND
1983	Live with signs of IHN	16/79 (20.3)	9.3×10^4
	Live with lesions on peduncle or tail	12/79 (15.2)	ND
	Live without signs of IHN	ND	ND
	Dead with signs of IHN	1/79 (1.3)	7.0×10^4
	Dead without signs of IHN	ND	ND
1984	Live with signs of IHN	ND	ND
	Live with lesions on peduncle or tail	ND	ND
	Live without signs of IHN	4/181 (2.2)	3.5×10^4
	Dead with signs of IHN	6/39 (15.4)	4.2×10^5
	Dead without signs of IHN	1/39 (2.6)	2.3×10^4

a/ Number positive/number examined

b/ Percent positive

noted: Dorsal fin, 19 (23.5%); caudal peduncle, 33 (40.7%); adipose fin, 8 (9.8%); anal fin, 5 (6.2%); pelvic fin, 6 (7.4%); pectoral fin, 6 (7.4%); and head, 4 (5.0%).

1983

Of a total population of 235,233 smolts, 535 (0.2%) abnormal or dead smolts were observed. The viral assay results for 79 smolts examined are provided in Table 2. Of these, live fish with signs of the disease comprised 94.9% of the sample; 88.0% of these exhibited lesions or fungusing of the caudal fin. Fungal infection was usually associated with IHN-positive fish. Of these selected live smolts, 21.3% were positive for IHNV. In the 4 dead smolts sampled, a single smolt with signs of the disease had 7.0×10^4 pfu IHNV/g of pooled kidney, liver, and spleen tissue, and one of the remaining 3 fish also was virus-positive. The mean virus intensity of the pooled kidney, spleen, and liver tissues taken from 7 live smolts with signs of the disease was 9.3×10^4 pfu/g (SD 8.7×10^1).

1984

In 1984, 938 (0.2%) live or dead smolts showing abnormalities were removed from the total number of smolts (419,797) that went through the fyke net. It was not determined how many actually had IHN, but all had representative signs of the disease. The intensity of IHNV was determined for some of these fish. The virus-causing IHN was isolated from pooled kidney, liver, and spleen tissues from 4 of an additional 181 (2.2%) normal-appearing smolts that were randomly sampled. The geometric mean viral intensity of these 4 fish was 3.5×10^4 pfu/g (*See* Table 2). Although the sample size was small, if the 2.2% were added to the 0.2%, as many as 10,000 fish may have been affected.

Of the 7 dead smolts examined and found to contain the virus, the geometric mean viral intensity was 2.8×10^5 pfu/g. Six smolts had signs of IHN; the one dead smolt with the virus but without signs of the disease had a level of 2.3×10^4 pfu/g present in the pooled organs. Hemorrhage at the base of the fins or erosion of the caudal peduncles were

prominent signs. Erosion of the caudal peduncle was often so pronounced that the caudal fin had been nearly severed; smolts without caudal fins were observed.

Twenty-six of 32 (81.3%) live smolts with clinical signs of the disease had IHNV and a mean viral intensity of 1.2×10^5 pfu/g. Twelve of the live fish with clinical signs had eroded caudal peduncles or tails; all of these had the virus. Some of these smolts had external fungus as well. Examination of the tail and caudal tissues from 5 of these 12 indicated a mean IHN viral intensity of these tissues to be almost 4 $\log_{10}$. The mean viral intensity of visceral organ tissue in four of these fish that were individually tested resulted in 4.7×10^4 pfu/g, while the lesion tissue had a mean titer of 7.0×10^3 pfu/g. The visceral organs from the fifth fish were not tested. The small sample size does not support a good statistical comparison, but in the four assayed fish, there was no statistically apparent difference in viral intensities between pooled kidneys, livers, and spleens compared with lesion tissues. All but one of the organ samples were numerically higher (by possibly 0.8 to 1.8 $\log_{10}$) than lesion tissues.

These strengthen the perspective that the caudal erosions observed may not be coincidental, but a secondary result of the IHN disease process. However, virus levels of the pooled organ tissues from the four randomly sampled fish were not significantly different from those of the same type of sample in live smolts (26) showing signs of the disease: $P < 0.05$ using the "t" test for two means on $\log_{10}$-transformed data. Samples that were similar to the 6 dead smolts containing virus and having signs of the disease were compared to those for the 26 fish. There was no significant difference in intensity ($P < 0.05$) using the "t" test for two means on $\log_{10}$-transformed data.

Pattern of Virus Detection

The proportion of abnormal and dead smolts infected with IHNV appeared to increase with time throughout the migration period. It was apparent that much of the mortality had historically occurred in the last part of the run (Burke and Grischkowsky 1984). The examination of data for 1982, 1983, and 1984 indicated that 15 June was near the commencement of the

mortality. By that date, approximately 60% (1982, 59.0%; 1983, 69.4%; 1984, 54.6%) of the smolts had migrated. We collected smolts by 15 June as follows: 13 for 1982, 27 for 1983, and 2 for 1984. The number of smolts collected after that date follow: 6 for 1982, 53 for 1983, and 44 for 1984. Over one-fourth (25.1%) of the 223 abnormal or dead sockeye salmon smolts collected after 15 June had IHNV in the pooled spleen, liver, and kidney tissues. Only 7.8% of the 42 smolts collected earlier had the virus.

In 1984 isolation of IHNV occurred from one of the four normal-appearing fish (of 181) sampled by 15 June; for the remaining three fish, it occurred later. Water-temperature data usually collected between 1030 and 1300 hours from 10 June through 3 July during these years are provided in Table 3. Table 3 also shows that the percentage of smolts that passed the weir is similar for 1982, 1983, and 1984 between 15 June and 3 July.

Infected Adult Salmon

Sockeye salmon ovarian fluids collected from 1981 through 1983 resulted in geometric mean IHNV titers of 10^4 to 10^5 pfu/ml, and for those years the means were within 1.8 $\log_{10}$ of each other. The titer range varied widely, particularly the first year with a large standard deviation. Prevalences between years also differed greatly at 26.8%, 79.7%, and 100% in 1983, 1981, and 1982, respectively (Figure 1).

DISCUSSION

The number of sockeye salmon smolt mortalities and clinically diseased fish declined from 2.5% in 1980 and 0.6% in 1981 (Burke and Grischkowsky 1984) to the 0.3% in 1982 and 0.2% for both 1983 and 1984 (Figure 2). The first major fish transplants were in 1983 at over 1,092,000 with further increases of 12% in 1984 and 61% in 1985.

Table 3. Water temperatures and numbers of sockeye salmon smolts with infectious hematopoietic necrosis virus during sockeye salmon smolt enumeration at Hidden Creek for 1982, 1983, and 1984.[a/]

	1982		1983		1984	
Day/Month	°C	% smolt at weir	°C	% smolt at weir	°C	% smolt at weir
10/6	11	21.2		48.2	11	37.0
11/6	10		...		15	
12/6	9		11		15	
13/6	9		...		...	
14/6	10		11		11	
15/6	...	59.0	12	69.0	11	54.6
16/6	12		...		11	
17/6	12		12		12	
18/6	11		...		134	
19/6	...		13		14	
20/6	11	85.7		86.6	14	82.7
21/6	10		13		15	
22/6	...		15		14	
21-22/6						
23/6	...		13		12	
24/6	16		16		12	
25/6	...	92.5	14	91.0	12	90.5
26/6	16		14		13	
27/6	...		14		13	
28/6	12		14		12	
29/6	10		14		11	
30/6	10	96.0	...	95.0	12	95.1
1/7	10		15		12	
2/7	12		15		13	
3/7	12	96.6	...	97.2	14	97.0
1-3/7						

[a/] Temperatures listed were usually from a 1030 to 1300 h daily reading.

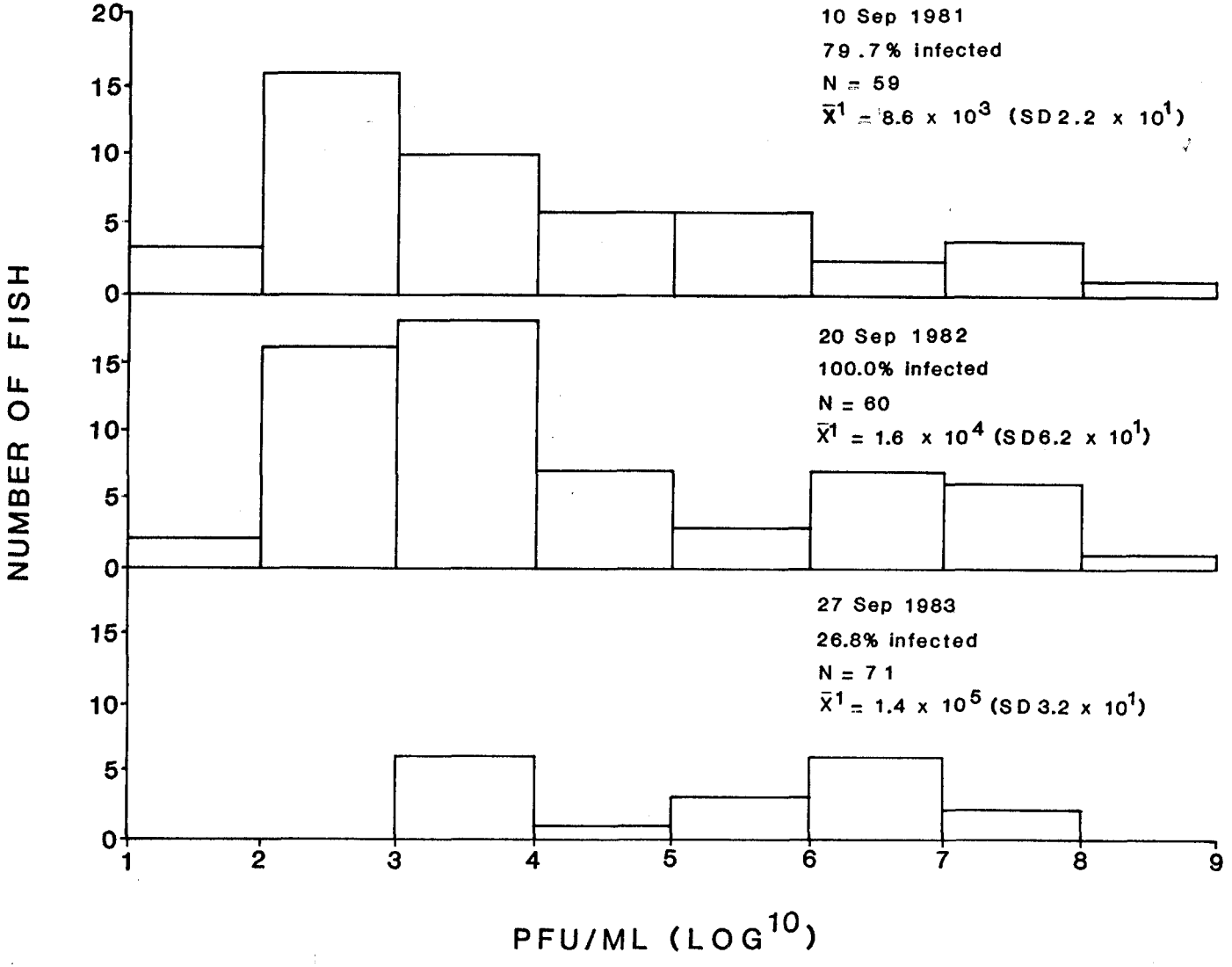

Figure 1. Concentration distribution of infectious hematopoietic necrosis virus in plaque-forming units (PFU) per milliliter of ovarian fluids in spawning or spawned sockeye salmon from Hidden Lake, 1981 through 1983.

1/ Geometric mean

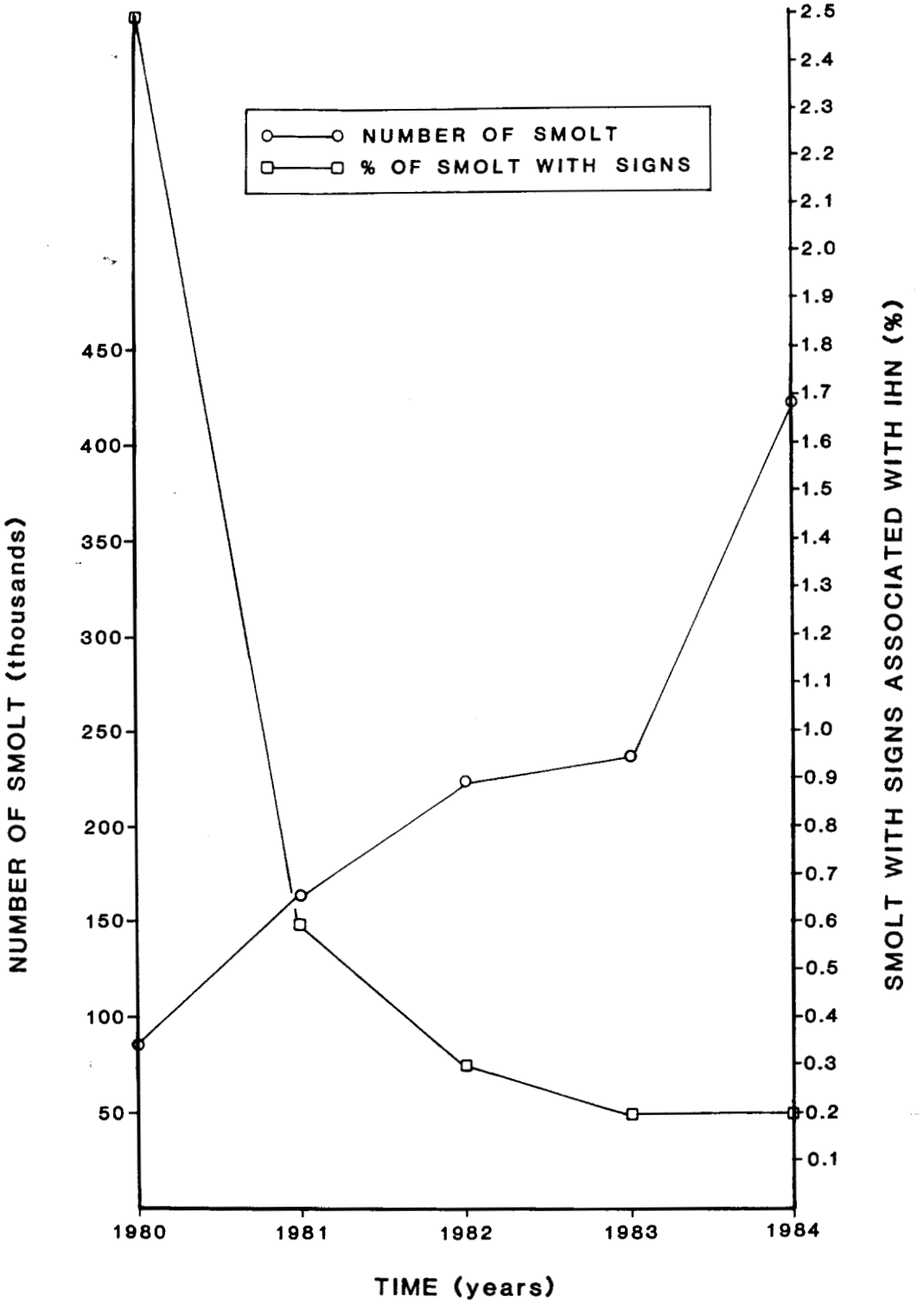

Figure 2. The total number of sockeye salmon smolts examined and the percentages having signs associated with infectious hematopoietic necrosis (IHN) for 1980 through 1984 at Hidden Creek.

Our study of ovarian fluids revealed prevalences of about 27% to 100% and viral intensities in individual fish from 1 to 8 $\log_{10}$ pfu/ml. From year to year, infection by IHNV in adult sockeye salmon varied most in prevalence and range in titers and least in overall geometric mean viral titer. The latter was within 1.8 $\log_{10}$.

The detection of IHNV in even a few live smolts without clinical signs of disease was an unusual observation in Alaska. These fish must have been early or convalescent cases. The virus levels in internal organs from the four grossly normal smolts were not significantly different than those of moribund fish with signs of IHN; however, to conclusively corroborate that finding, a larger sample size would have to be used. Numerically, half of the 26 clinically diseased fish had virus titers higher than the normal-appearing smolts.

Typically, the virus can only be isolated in fry during an epizootic and when adult fish spawn (Mulcahy et al. 1984). The detection of IHNV in fish without signs may indicate they were exposed to virus sufficient for transmission, additional mortality might have occurred downstream, and the carrier status might have been affected. The detection of the virus in a dead smolt without signs indicates they can be infected without showing external abnormalities. These determinations supplement the finding of IHN in normal-appearing live sockeye salmon smolts (Burke and Grischkowsky 1984) when anatomy was microscopically examined. Accordingly, the number of smolts infected with IHNV may be much greater than that observed at the weir. There is also no reason to believe IHN is occurring only at the weir site. Mortalities that go undetected may be occurring in Hidden Lake, downstream from the weir in Skilak Lake, or in the Kenai River.

Caudal peduncle and tail erosion was the most commonly observed gross abnormality after a daily tabulation for one week in 1982. All smolts with IHN-associated signs collected during the last three days of that period had IHNV. For all three years, caudal peduncle abnormalities have consistently been involved with this seasonal mortality. Findings of the virus in fish with that condition ranged from 19% to 100% in 1983 and 1984, respectively. The caudal peduncle and caudal fin erosions may be a secondary sequela to the IHN disease process.

We are in agreement with Burke and Grischkowsky (1984): The disease is probably predominant in the fish that emigrate during the late portion of the run. In this situation, IHNV, warm temperatures, and emigration all coincide at Hidden Creek.

Collection of viral data at Hidden Creek could be facilitated by using the association with caudal peduncle or caudal fin lesions. Sampling smolts only after 15 June would also reduce the cost. Moreover, sorting out smolts presumed to be infected would be easy for those people operating a weir.

Burke and Grischkowsky (1984) reported results similar to our findings of virus in brains of dead smolts, but not in those of live smolts showing signs. Contrary to Mulcahy et al. (1984), IHNV was routinely found in brains of virus-infected fish during this study. We found the virus concentration in brains, however, at 3 $\log_{10}$ lower than in internal organs. When virus was detected in our study, it was not at significantly different titers in the internal organs of normal-appearing infected smolts than in live or dead smolts with signs. The 12.5-km (7.8 mi) by 2.5-km (1.6 mi) lake is drained by Hidden Creek at the rate of approximately 0.1 m^3/s (Lorenz 1984). This very low drainage rate for Hidden Lake may help in viral transmission. The situation precipitating IHN among Hidden Lake smolts presents unique opportunities for investigating why the disease occurred every year during this study and if a density relationship exists between sockeye salmon IHN disease and viral prevalence.

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